Practitioner's Docket No. Lettuce

CHAPTER II

Preliminary Classification:

Proposed Class:

Subclass:

NOTE: "All applicants are requested to include a preliminary classification on newly filed patent applications. The preliminary classification, preferably class and subclass designations, should be identified in the upper right-hand corner of the letter of transmittal accompanying the application papers, for example 'Proposed Class 2, subclass 129.' " M.P.E.P., § 601, 7th ed.

TRANSMITTAL LETTER TO THE UNITED STATES ELECTED OFFICE (EO/US)

(ENTRY INTO U.S. NATIONAL PHASE UNDER CHAPTER II)

PCT GB99 04317 (INTERNATIONAL APPLICATION NO.)
December 16 1999 (INTERNATIONAL FILING DATE)
December 21 1998 (PRIORITY DATE CLAIMED)

Genetic Modification of composi[illegible]tae
TITLE OF INVENTION

F.M.A. Schepers C.M.P. Van Dun J.M. Pertrijs
APPLICANT(S)

Box PCT
Assistant Commissioner for Patents
Washington D.C. 20231
ATTENTION: EO/US

CERTIFICATION UNDER 37 C.F.R. § 1.10*
*(Express Mail label number is **mandatory**.)*
(Express Mail certification is optional.)

I hereby certify that this Transmittal Letter and the papers indicated as being transmitted therewith is being deposited with the United States Postal Service on this date June 20, 2001, in an envelope as "Express Mail Post Office to Addressee" Mailing Label Number EK831541056US, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

Susan E. Denman
(type or print name of person mailing paper)

Susan E. Denman
Signature of person mailing paper

***WARNING:** Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. § 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.*

***WARNING:** Each paper or fee filed by "Express Mail" **must** have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. § 1.10(b).*
*"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.*

NOTE: To avoid abandonment of the application, the applicant shall furnish to the USPTO, not later than 20 months from the priority date: (1) a copy of the international application, unless it has been previously communicated by the International Bureau or unless it was originally filed in the USPTO; and (2) the basic national fee (see 37 C.F.R. § 1.492(a)). The 30-month time limit may not be extended. 37 C.F.R. § 1.495.

WARNING: *Where the items are those which can be submitted to complete the entry of the international application into the national phase are subsequent to 30 months from the priority date the application is still considered to be in the international state and if mailing procedures are utilized to obtain a date the express mail procedure of 37 C.F.R. § 1.10 must be used (since international application papers are not covered by an ordinary certificate of mailing—See 37 C.F.R. § 1.8.*

NOTE: Documents and fees must be clearly identified as a submission to enter the national state under 35 U.S.C. § 371 otherwise the submission will be considered as being made under 35 U.S.C. § 111. 37 C.F.R. § 1.494(f).

I. Applicant herewith submits to the United States Elected Office (EO/US) the following items under 35 U.S.C. § 371:

a. ☒ This express request to immediately begin national examination procedures (35 U.S.C. § 371(f)).

b. ☒ The U.S. National Fee (35 U.S.C. § 371(c)(1)) and other fees (37 C.F.R. § 1.492) as indicated below:

2. Fees

CLAIMS FEE	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5) CALCULATIONS
[ ]*	TOTAL CLAIMS	21 −20=	1	× $18.00=	$ 18.00
	INDEPENDENT CLAIMS	1 −3=	0 0	× $80.00=	0
	MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $270.00	0
BASIC FEE**	☐ U.S. PTO WAS INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where an International preliminary examination fee as set forth in § 1.482 has been paid on the international application to the U.S. PTO: ☐ and the international preliminary examination report states that the criteria of novelty, inventive step (non-obviousness) and industrial activity, as defined in PCT Article 33(1) to (4) have been satisfied for all the claims presented in the application entering the national stage (37 C.F.R. § 1.492(a)(4)) ..... $100.00 ☐ and the above requirements are not met (37 C.F.R. § 1.492(a)(1)) ..... $690.00 X☐ U.S. PTO WAS NOT INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where no international preliminary examination fee as set forth in § 1.482 has been paid to the U.S. PTO, and payment of an international search fee as set forth in § 1.445(a)(2) to the U.S. PTO: ☐ has been paid (37 C.F.R. § 1.492(a)(2)) ..... $710.00 ☐ has not been paid (37 C.F.R. § 1.492(a)(3)) ..... $1000.00 X☐ where a search report on the international application has been prepared by the European Patent Office or the Japanese Patent Office (37 C.F.R. § 1.492(a)(5)) $860.00				860.00
	Total of above Calculations				=
SMALL ENTITY	Reduction by 1/2 for filing by small entity, if applicable. Affidavit must be filed also. (note 37 C.F.R. § 1.9, 1.27, 1.28)				–
	Subtotal				
	Total National Fee				$ 878.00
	Fee for recording the enclosed assignment document $40.00 (37 C.F.R. § 1.21(h)). (See Item 13 below). See attached "ASSIGNMENT COVER SHEET".				
TOTAL	Total Fees enclosed				$ 878.00

*See attached Preliminary Amendment Reducing the Number of Claims.

☐ Attached is a ☐ check ☐ money order in the amount of $ _____

☐ Authorization is hereby made to charge the amount of $ _____

☐ to Deposit Account No. _____

☒ to Credit card as shown on the attached credit card information authorization form PTO-2038.

***WARNING:** Credit card information should not be included on this form as it may become public.*

☒ Charge any additional fees required by this paper or credit any overpayment in the manner authorized above.

A duplicate of this paper is attached.

***WARNING:** "To avoid abandonment of the application the applicant shall furnish to the United States Patent and Trademark Office not later than the expiration of 30 months from the priority date: * * * (2) the basic national fee (see § 1.492(a)). The 30-month time limit may not be extended." 37 C.F.R. § 1.495(b).*

***WARNING:** If the translation of the international application and/or the oath or declaration have not been submitted by the applicant within thirty (30) months from the priority date, such requirements may be met within a time period set by the Office. 37 C.F.R. § 1.495(b)(2). The payment of the surcharge set forth in § 1.492(e) is required as a condition for accepting the oath or declaration later than thirty (30) months after the priority date. The payment of the processing fee set forth in § 1.492(f) is required for acceptance of an English translation later than thirty (30) months after the priority date. Failure to comply with these requirements will result in abandonment of the application. The provisions of § 1.136 apply to the period which is set. Notice of Jan. 3, 1993, 1147 O.G. 29 to 40.*

3. ☒ A copy of the International application as filed (35 U.S.C. § 371(c)(2)):

NOTE: Section 1.495 (b) was amended to require that the basic national fee and a copy of the international application must be filed with the Office by 30 months from the priority date to avoid abandonment. "The International Bureau normally provides the copy of the international application to the Office in accordance with PCT Article 20. At the same time, the International Bureau notifies applicant of the communication to the Office. In accordance with PCT Rule 47.1, that notice shall be accepted by all designated offices as conclusive evidence that the communication has duly taken place. Thus, if the applicant desires to enter the national stage, the applicant normally need only check to be sure the notice from the International Bureau has been received and then pay the basic national fee by 30 months from the priority date." Notice of Jan. 7, 1993, 1147 O.G. 29 to 40, at 35-36. See item 14c below.

a. ☒ is transmitted herewith.

b. ☐ is not required, as the application was filed with the United States Receiving Office.

c. ☐ has been transmitted

i. ☐ by the International Bureau.
Date of mailing of the application (from form PCT/1B/308):
_____.

ii. ☐ by applicant on _____ *(Date)*

4. ☒ A translation of the International application into the English language (35 U.S.C. § 371(c)(2)):

a. ☐ is transmitted herewith.

b. ☒ is not required as the application was filed in English.

c. ☐ was previously transmitted by applicant on _____. *(Date)*

d. ☐ will follow.

5. ☒ Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. § 371(c)(3)):

NOTE: *The Notice of January 7, 1993 points out that 37 C.F.R. § 1.495(a) was amended to clarify the existing and continuing practice that PCT Article 19 amendments must be submitted by 30 months from the priority date and this deadline may not be extended. The Notice further advises that: "The failure to do so will not result in loss of the subject matter of the PCT Article 19 amendments. Applicant may submit that subject matter in a preliminary amendment filed under section 1.121. In many cases, filing an amendment under section 1.121 is preferable since grammatical or idiomatic errors may be corrected." 1147 O.G. 29-40, at 36.*

a. ☐ are transmitted herewith.

b. ☐ have been transmitted

i. ☐ by the International Bureau.
Date of mailing of the amendment (from form PCT/1B/308):

_____.

ii. ☐ by applicant on _____. *(Date)*

c. ☒ have not been transmitted as

i. ☒ applicant chose not to make amendments under PCT Article 19.
Date of mailing of Search Report (from form PCT/ISA/210.):

_____.

ii. ☐ the time limit for the submission of amendments has not yet expired. The amendments or a statement that amendments have not been made will be transmitted before the expiration of the time limit under PCT Rule 46.1.

6. ☒ A translation of the amendments to the claims under PCT Article 19 (38 U.S.C. § 371(c)(3)):

a. ☐ is transmitted herewith.

b. ☐ is not required as the amendments were made in the English language.

c. ☒ has not been transmitted for reasons indicated at point 5(c) above.

7. ☒ A copy of the international examination report (PCT/IPEA/409)

☒ is transmitted herewith.

☐ is not required as the application was filed with the United States Receiving Office.

8. ☒ Annex(es) to the international preliminary examination report

a. ☒ is/are transmitted herewith.

b. ☐ is/are not required as the application was filed with the United States Receiving Office.

9. ☒ A translation of the annexes to the international preliminary examination report

a. ☐ is transmitted herewith.

b. ☒ is not required as the annexes are in the English language.

10.☒ An oath or declaration of the inventor (35 U.S.C. § 371(c)(4)) complying with 35 U.S.C. § 115

a. ☐ was previously submitted by applicant on _____ Date

b. ☒ is submitted herewith, and such oath or declaration

i. ☐ is attached to the application.

ii. ☐ identifies the application and any amendments under PCT Article 19 that were transmitted as stated in points 3(b) or 3(c) and 5(b); and states that they were reviewed by the inventor as required by 37 C.F.R. § 1.70.

c. ☒ will follow.

II. Other document(s) or information included:

11.☒ An International Search Report (PCT/ISA/210) or Declaration under PCT Article 17(2)(a):

a. ☐ is transmitted herewith.

b. ☐ has been transmitted by the International Bureau. Date of mailing (from form PCT/IB/308): _____.

c. ☐ is not required, as the application was searched by the United States International Searching Authority.

d. ☒ will be transmitted promptly upon request.

e. ☐ has been submitted by applicant on _____ Date

12. ☒ An Information Disclosure Statement under 37 C.F.R. §§ 1.97 and 1.98:

a. ☐ is transmitted herewith.

Also transmitted herewith is/are:

☐ Form PTO-1449 (PTO/SB/08A and 08B).

☐ Copies of citations listed.

b. ☒ will be transmitted within THREE MONTHS of the date of submission of requirements under 35 U.S.C. § 371(c).

c. ☐ was previously submitted by applicant on _____. Date

13. ☐ An assignment document is transmitted herewith for recording.

A separate ☐ "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or ☐ FORM PTO 1595 is also attached.

14. ☐ Additional documents:
    a. ☐ Copy of request (PCT/RO/101)
    b. ☐ International Publication No. _____
        i. ☐ Specification, claims and drawing
        ii. ☐ Front page only
    c. ☐ Preliminary amendment (37 C.F.R. § 1.121)
    d. ☐ Other

15. ☒ The above checked items are being transmitted
    a. ☒ before 30 months from any claimed priority date.
    b. ☐ after 30 months.

16. ☐ Certain requirements under 35 U.S.C. § 371 were previously submitted by the applicant on _____, namely:

AUTHORIZATION TO CHARGE ADDITIONAL FEES

WARNING: *Accurately count claims, especially multiple dependant claims, to avoid unexpected high charges if extra claims are authorized.*

NOTE: *"A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. § 1.136(a)(3).*

NOTE: *"Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).*

☐ Please charge, in the manner authorized above, the following additional fees that may be required by this paper and during the entire pendency of this application:

☐ 37 C.F.R. § 1.492(a)(1), (2), (3), and (4) (filing fees)

WARNING: *Because failure to pay the national fee within 30 months without extension (37 C.F.R. § 1.495(b)(2)) results in abandonment of the application, it would be best to always check the above box.*

☐ 37 C.F.R. § 1.492(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 C.F.R. § 1.492(d)), it might be best not to authorize the PTO to charge additional claim fees, except possible when dealing with amendments after final action.

☐ 37 C.F.R. § 1.17 (application processing fees)

☐ 37 C.F.R. § 1.17(a)(1)–(5) (extension fees pursuant to § 1.136(a).

☐ 37 C.F.R. § 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. § 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 C.F.R. § 1.311(b).

NOTE: 37 C.F.R. § 1.28(b) requires "Notification of any change in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying . . . issue fee." From the wording of 37 C.F.R. § 1.28(b): (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

☐ 37 C.F.R. § 1.492(e) and (f) (surcharge fees for filing the declaration and/or filing an English translation of an International Application later than 30 months after the priority date).

Dana Rewoldt

SIGNATURE OF PRACTITIONER

Reg. No.: 33762

Dana Rewoldt

(type or print name of practitioner)

Tel. No.: (515) 685-5113

2369 330th ST. Box 500

P.O. Address

Customer No.:

Slater Iowa 50244

Practitioner's Docket No. Lettuce ***PATENT***

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

RE:	Patent Application for Schepers	:	Date:	November 9, 2001
Serial No.:	09/868,744	:	Group No.:	
Filed:		:	Examiner:	
For:	Genetic Modification of Compositae	:		

Box Sequence
Assistant Commissioner for Patents
Washington, DC 20231

SUBMISSION OF "SEQUENCE LISTING," COMPUTER READABLE COPY, AND/OR AMENDMENT PERTAINING THERETO FOR BIOTECHNOLOGY INVENTION CONTAINING NUCLEOTIDE AND/R AMINO ACID SEQUENCE

1. This replies to the Office Letter dated July 27, 2001.

IDENTIFICATION OF PERSON MAKING STATEMENT

2. I, Dana Rewoldt, state the following:

ITEMS BEING SUBMITTED:

3. Submitted herewith are:

   A. "Sequence Listing(s)" for the nucleotide and/or amino acid sequence(s) in this application. Each "Sequence Listing" is assigned a separate identifier as required in 37 C.F.R. § 1.82(c) and 37 C.F.R. §§ 1.822 and 1.823.

   B. An amendment to the description and/or claims, wherein reference is made to the sequence by use of the assigned identifier, as required in 37 C.F.R. § 1.821(d).

   C. A copy of each "Sequence Listing" submitted for this application in computer readable form, in accordance with the requirements of 37 C.F.R. §§ 1.821(e) and 1.824.

D. A statement that the content of each "Sequence Listing" submitted and each computer readable copy are the same, as required in 37 C.F.R. § 1.821(g).

E. Because this submission is made in fulfilling the requirement under 37 C.F.R. § 1.821(g), a statement that the submission includes no new matter.

STATEMENT THAT "SEQUENCE LISTING" AND COMPUTER READABLE COPY ARE THE SAME AND/OR THAT PAPERS SUBMITTED INCLUDES NO NEW MATTER

4. I hereby state that each computer readable form submitted in this application is the same as the "Sequence Listing" to which it is indicated to relate.

5. All papers accompanying this submission introduce no new matter in the application submitted to the USPTO by the International Bureau.

STATUS

6. Applicant is other than a small entity.

AMENDMENT TO DESCRIPTION

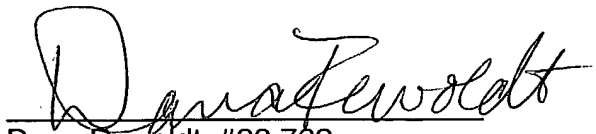
7. On page 3, line 15 was amended as follows:

Figure 3 gives Seq. ID No. 1 which is the DNA sequence of the actin2 (ACT2) promoter derived from *Arabidopsis*.

8. On page 10, lines 4 and 5 were amended as follows:

consisting of primer 1 (5'-GC AAGCTT ATT ATG ATC TCA AAT ACA TTG-3'), Seq. ID No. 2, and primer 2 (5'-GC GGATCC TTT ATG AGC TGC AAA CAC AC-3'), Seq. ID No. 3. Primer 1 contains.

Respectfully submitted,



Dana Rewoldt, #33,762
Advanta USA, Inc.
2369 330th Street, Box 500
Slater, IA 50244
Tel: (515) 685-5201
Fax: (515) 685-5072

CERTIFICATE OF MAILING UNDER 37 C.F.R. 1.8

I hereby certify that the above Submission of "Sequence Listing," Computer Readable Copy and/or Amendment Pertaining Thereto for Biotechnology Invention Containing Nucleotide and/or Amino Acid Sequence, two declarations, and attached postcard is being deposited in with the U.S. Postal Service as first-class mail in an envelope addressed to Box Sequence, Assistant Commissioner for Patents, Washington, DC 20231, on November 9, 2001.

Ruth Anderson

Kapelle, June 19th 2001

U.S. Patent and Trademark Office
BOX PCT
Assistant Commissioner for Patents
Washington, DC 20231
U.S.A.

Re: Patent Application
International application No: PCT/GB99/04317
Filed: December 16 1999
For: Genetic Modification of Compositae

Date: June 19th 2001
Art Unit:
Examiner
Action: preliminary Amendment

Dear Sirs,

Please amend the application according to the attached paperwork.
The next three pages identified as Preliminary Amendment show the changes made to the claims.
The last three claim sheets "Amended Sheet" show the claims in corrected form.

Sincerely,

Garst Seed Company
Dana Rewoldt, #33,762
Patent Attorney
P.O. Box 500
Slater, Iowa 50244

CERTIFICATION UNDER 37 C.F.R § 1.10 *
(Express Mail label number is mandatory)
(Express Mail certification is optional)

I hereby certify that this preliminary Amendment is being transmitted therewith are being deposited with the United States Postal Services on this date, June 19th 2001, in an envelope as "EXPRESS MAIL POST OFFICE TO ADDRESSEE" Mailing Label Number EK831541056 4S addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Sue Denmon

Signature of person mailing paper

09868744 .010902
09/868744
531 Rec'd PC 20 JUN 2001

Kapelle, June 19th 2001

U.S. Patent and Trademark Office
BOX PCT
Assistant Commissioner for Patents
Washington, DC 20231
U.S.A.

Re: Patent Application
International application No: PCT/GB99/04317
Filed: December 16 1999
For: Genetic Modification of Compositae

Date: June 19th 2001
Art Unit:
Examiner
Action: preliminary Amendment

Dear Sirs,

Please amend the application according to the attached paperwork.

Insert 3
The first pages identified as Preliminary Amendment show the changes made to the claims. The last three claim sheet "amended sheet" should show the claims in corrected form.

Sincerely,

Garst Seed Company
Dana Rewoldt, #33,762
Patent Attorney
P.O. Box 500
Slater, Iowa 50244

CERTIFICATION UNDER 37 C.F.R § 1.10 *
(Express Mail label number is mandatory)
(Express Mail certification is optional)

I hereby certify that this preliminary Amendment is being transmitted therewith are being deposited with the United States Postal Services on this date, June 19th 2001, in an envelope as "EXPRESS MAIL POST OFFICE TO ADDRESSEE" Mailing Label Number EK831541056US addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Sue Denmon

Sue Denmon

Signature of person mailing paper

#3.

BOX PCT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: F. Schepers et al. Attorney Docket No.: Lettuce

Int'l. Application No.: PCT/GB99/04317 Int'l Filing Date: December 16, 1999

U.S. Application No.: 09/868,744

Title: GENETIC MODIFICATION OF COMPOSITAE

RESPONSE TO NOTIFICATION OF DEFECTIVE RESPONSE AND SUBSTITUTE PRELIMINARY AMENDMENT

Seattle, Washington 98101

February 14, 2003

TO THE COMMISSIONER FOR PATENTS:

INTRODUCTORY COMMENTS

Sequence Listing

In response to the Notification of Defective Response mailed January 30, 2003, transmitted herewith is a sequence listing in printed and computer-readable formats (37 C.F.R. § 1.821(e)). The paper and computer-readable copies of the sequence listing are the same (37 C.F.R. § 1.821(f)) and do not contain new matter (37 C.F.R. § 1.821(g)). Entry of the sequence listing into the application is requested. Also transmitted herewith is a copy of the 371 Formalities Letter.

Substitute Preliminary Amendment

A preliminary Amendment was initially filed in this application on July 31, 2002. It has subsequently come to applicants' attention that amendments to the specification proposed in that Amendment were presented in an improper format.

It is therefore requested that the Amendment filed July 31, 2002, not be entered and that the following amendments to the specification be substituted and entered in this application.

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS PLLC
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

AMENDMENTS TO THE SPECIFICATION

Please rewrite the first full paragraph on page 9 to read as follows:

A further experiment was done to illustrate the use of the act2 promoter to drive other genes besides GUS. As the act2/GUS construct pVDH380 (Figure 1) contains 19 condons of the actin2 gene it was decided to modify the promoter by PCR using a primer combination, see Seq. ID No. 2 and Seq. ID No. 3, which generates a unique restriction site at the act2 transcription start. This modified promoter was fused to the OXOX gene and inserted into a binary vector, as described below.

Please rewrite the paragraph spanning pages 9 and 10 to read as follows:

Figure 3 shows the nucleotide sequence of the Actin 2 promoter region, see Seq. ID No. 1. The sequence corresponding to the forward primer, see Seq. ID No. 2 (bold, **Gothic typeface**), as well as to the complementary sequence, see Seq. ID No. 3 (bold underlined), of the backward primer are indicated. The start condon of the Actin 2 gene, ATG is given in bold capitals. In addition, the composition of the forward and backward primers are given. We used a primerset consisting of primer 1 (5'-GC AAGCTT ATT ATG ATC TCA AAT ACA TTG-3') and primer 2 (5'-GC GGATCC TTT ATG AGC TGC AAA CAC AC-3'). Primer 1 contains after the first two nucleotides a HindIII restriction recognition site and subsequently a nucleotide sequence identical to the nucleotide sequence located from position 1358 to position 1379 upstream from the ATG-start codon (see Figure 3). Primer 2 contains after the first two nucleotides a BamHI restriction recognition site and subsequently 20 nucleotides complementary from position 3 to position 22 upstream from the start condon. The DNA fragment which was obtained after amplification was digested with HindIII and BamHI and inserted in the vector pVDH478. pVDH478 is a binary vector containing between the left and right border the NPTII gene, flanked upstream by the nopaline synthase promoter and downstream by the nopaline

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS PLLC
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

synthase poly(A)-signal. It also contains the coding region of the oxalate oxidase gene (OxOx) with its own poly(A)-signal which is derived from wheat (for more information on the OxOx gene, including sequence data, see PCT Publication WO92/14824). The resulting vector was called pVDH641. A physical map of pVDH641 is shown in Figure 2. In this Figure, annotations in common with Figure 1 have the same meaning as in that Figure. Additionally, 'ToxOx' indicates the oxalate oxidase terminator and 'OxOx' indicates the oxalate oxidase gene. The main restriction enzymes are indicated.

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CHRISTENSEN O'CONNOR JOHNSON KINDNESS PLLC
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

REMARKS

Entry of the substitute sequence listing and foregoing amendments to the specification and favorable substantive examination of the application is requested.

Respectfully submitted,

CHRISTENSEN O'CONNOR
JOHNSON KINDNESS PLLC

Dennis K. Shelton
Registration No. 26,997
Direct Dial No. 206.695.1718

DKS:cj

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS PLLC
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

The promoter of the actin2 (ACT2) gene derived from *Arabidopsis* has been shown to drive the beta-glucuronidase (GUS) gene in transgenic *Arabidopsis* plants to relatively high levels in vegetative tissues. In the inflorescence a development factor seemed to be involved causing a more differentiated expression pattern. This reported by An, Meagher *et al.* (1996), The Plant Journal 10, 107-121: the same reference the DNA sequence of the actin-2 promoter (p. 109).

The invention will be further described with reference to the drawings, in which:

Figure 1 is a map of a construct pVDH380 comprising the promoter of the actin2 (ACT2) gene derived from *Arabidopsis* arranged to drive the beta-glucuronidase (GUS) gene.

Figure 2 is a map of a construct pVDH641 comprising the promoter of the actin2 (ACT2) gene derived from *Arabidopsis* arranged to drive OXOX gene of wheat;

Figure 3 gives Seq. ID No. 1 which is the DNA sequence of the actin2 (ACT2) promoter derived from *Arabidopsis*.

Actin is a fundamental cytoskeletal component essential to nearly all eukaryotic cells, in which it forms microfilament structures. There are large families of plant actin genes, with greater diversity than corresponding animal genes. The actin 2 gene promoter is a constitutive promoter to be found in most plants. We particularly prefer to use the actin 2 gene promoter obtained from *Arabidopsis thaliana*: though corresponding actin 2 promoters can readily be isolated from other sources, particularly other plants, and used for the same purpose.

The DNA sequence which expresses RNA may be of two main kinds: either a sequence which expresses mRNA which is translated into protein, or a sequence which

complementary sequence (bold underlined) of the backward primer are indicated. The start codon of the Actin 2 gene, ATG is given in bold capitals. In addition, the composition of the forward and backward primers are given. We used a primerset consisting of primer 1 (5'-GC AAGCTT ATT ATG ATC TCA AAT ACA TTG-3'), (Seq. ID No. 2), and primer 2 (5'-GC GGATCC TTT ATG AGC TGC AAA CAC AC-3'), (Seq. ID No. 3). Primer 1 contains after the first two nucleotides a HindIII restriction recognition site and subsequently a nucleotide sequence identical to the nucleotide sequence located from position 1358 to position 1379 upstream from the ATG-start codon (see Figure 3). Primer 2 contains after the first two nucleotides a BamHI restriction recognition site and subsequently 20 nucleotides complementary from position 3 to position 22 upstream from the start codon. The DNA fragment which was obtained after amplification was digested with HindIII and BamHI and inserted in the vector pVDH478. pVDH478 is a binary vector containing between the left and right border the NPTII gene, flanked upstream by the nopaline synthase promoter and downstream by the nopaline synthase poly(A)-signal. It also contains the coding region of the oxalate oxidase gene (OxOx) with its own poly(A)-signal which is derived from wheat (for more information on the OxOx gene, including sequence data, see PCT Publication WO92/14824). The resulting vector was called pVDH641. A physical map of pVDH641 is shown in Figure 2. in this Figure, annotations in common with Figure 1 have the same meaning as in that Figure. Additionally, 'TOxOx' indicates the oxalate oxidase terminator, and 'OxOx' indicates the oxalate oxidase gene. The main restriction enzymes are indicated.

Sequence analysis confirmed that the ACT2 promoter region had been inserted without any mutation having occurred during the PCR amplification. OxOx activity can be measured in a histochemical assay using oxalate which is converted by the enzyme into a purple dye. The act2/OXOX

The promoter of the actin2 (ACT2) gene derived from *Arabidopsis* has been shown to drive the beta-glucuronidase (GUS) gene in transgenic *Arabidopsis* plants to relatively high levels in vegetative tissues. In the inflorescence a development factor seemed to be involved causing a more differentiated expression pattern. This reported by An, Meagher *et al.* (1996), The Plant Journal 10, 107-121: the same reference the DNA sequence of the actin-2 promoter (p. 109).

The invention will be further described with reference to the drawings, in which:

Figure 1 is a map of a construct pVDH380 comprising the promoter of the actin2 (ACT2) gene derived from *Arabidopsis* arranged to drive the beta-glucuronidase (GUS) gene.

Figure 2 is a map of a construct pVDH641 comprising the promoter of the actin2 (ACT2) gene derived from *Arabidopsis* arranged to drive OXOX gene of wheat;

Figure 3 gives Seq. ID No. 1 which is the DNA sequence of the actin2 (ACT2) promoter derived from *Arabidopsis*.

Actin is a fundamental cytoskeletal component essential to nearly all eukaryotic cells, in which it forms microfilament structures. There are large families of plant actin genes, with greater diversity than corresponding animal genes. The actin 2 gene promoter is a constitutive promoter to be found in most plants. We particularly prefer to use the actin 2 gene promoter obtained from *Arabidopsis thaliana*: though corresponding actin 2 promoters can readily be isolated from other sources, particularly other plants, and used for the same purpose.

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Preliminary Amendment 12
PCT GB99/04317

WE CLAIM:

1. A method of producing a genetically-modified *Compositae* plant in which the expression of the modified gene has a reduced tendency to silencing, which comprises transforming the plant with a heterologous DNA construct including a DNA sequence adapted to express RNA in the plant under the control of the actin2 (ACT2) gene promoter.

2. A method as claimed in claim 1 in which the ACT2 gene promoter is derived from *Arabidopsis thaliana*.

3. A method as claimed in claim 2 in which the ACT2 gene promoter has substantially the sequence shown in Figure 3.

4. (Once Amended) A method as claimed in [any of] claim[s] 1[-3] in which the RNA to be expressed in the plant codes for the production of a heterologous protein.

5. (Once Amended) A method as claimed in [any of] claim[s] 1[-3] in which the RNA to be expressed in the plant codes for the production of a homologous protein.

6. (Once Amended) A method as claimed in [any of] claim[s] 1[-3] in which the RNA to be expressed in the plant inhibits the production of a homologous protein.

7. A method as claimed in claim 6 in which the RNA to be expressed in the plant is antisense to DNA coding for a homologous protein.

8. (Once Amended) A method as claimed in [any of] claim[s] 1[-7] in which the plant is lettuce or sunflower.

9. Genetically-modified *Compositae* plant cells that may be produced by the process of claim 1 comprising a heterologous DNA construct including a DNA sequence adapted to express RNA in the plant under the control of the actin2 (ACT2) gene promoter.

10. A plant cell as claimed in claim 9 adapted to express RNA that produces recombinant heterologous protein in the cell.

11. A plant cell as claimed in claim 10 in which the heterologous protein is an insecticidal, fungicidal or antiviral protein, or one conferring herbicide resistance.

12. A plant cell as claimed in claim 11 in which the DNA construct is adapted to express the oxox gene.

13. A plant cell as claimed in claim 9 adapted to express RNA that produces recombinant homologous protein in the cell.

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15. A plant cell as claimed in claim 12 in which the RNA to be expressed in the plant is antisense to DNA coding for a homologous protein.

Preliminary Amendment

16. A vector useful in the process of claim 1 which comprises a DNA construct including a DNA sequence adapted to express RNA in a plant under the control of the actin2 (ACT2) gene promoter, the DNA sequence comprising the gus gene or the oxox gene.

17. (Once Amended) *Compositae* plants comprising cells claimed in [any of] claim[s] 9[-15].

18. Plants as claimed in claim 17 which are lettuce.

19. Plants as claimed in claim 17 which are sunflower.

20. (Once Amended) A plant claimed in [any of] claim[s] 17[-19] which is adapted to express the oxox gene and is resistant to sclerotinia.

21. (Once Amended) A plant claimed in [any of] claim[s] 17[-19] which is adapted to express a heterologous gene conferring herbicide resistance.

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Amended Sheet

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Amended Sheet

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17. *Compositae* plants comprising cells claimed in claim 9.

18. Plants as claimed in claim 17 which are lettuce.

19. Plants as claimed in claim 17 which are sunflower.

20. A plant claimed in claim 17 which is adapted to express the oxox gene and is resistant to sclerotinia.

21. A plant claimed in claims 17 which is adapted to express a heterologous gene conferring herbicide resistance.

Amended Sheet

TITLE: GENETIC MODIFICATION OF *COMPOSITAE*

The present invention relates to the genetic modification of plants of the family *Compositae*, in particular lettuce (*Lactuca sativa*) and sunflower (*Helianthus annuus*).

Genetic modification of plants is now well established as an experimental technique, and increasingly such plants are being used in agriculture in different parts of the world. The technique offers a number of advantages. The introduction of heterologous genes allows the plant to produce heterologous proteins with functions that the plant does not normally possess. For example, introduction of the gene for production of the *Bacillus thuringiensis* (Bt) insecticidal protein renders the plant toxic to insects and protects it against insect attack Similarly genes may be introduced which protect against the attack of fungal and viral diseases. It is also possible by genetic modification to control the expression of homologous genes which the plant already possesses. Thus by inserting extra copies of homologous genes under the control of a suitable promoter, the expression of the protein produced by such genes can be up-regulated. Correspondingly, down-regulation may be induced by means such as anti-sense technology, in which an inverted copy of the homologous gene is inserted in the plant. Expression of the inverted gene produces antisense RNA, which inhibits the expression of the natural gene. In this way, for example, ripening of fruit such as tomatoes has been delayed by inhibiting the action of the polygalacturonase gene.

The genetic modification of plants in this way offers many potential benefits to farmers, consumers and the environment To farmers it offers the opportunity to avoid the labour and expense of chemical sprays; for consumers it can provide cheaper food of higher quality; it can protect the environment both by raising productivity (thereby

reducing pressure on agricultural land) and by reducing the amount of chemical pesticide introduced into the ecosphere.

A number of methods for transforming crop plants, both monocotyledons and dicotyledons, are now well-known. They include, for example, the ballistic method (the 'gene gun') in which heavy metal pellets, for example of gold or tungsten, are coated with DNA and fired into plant cells: and the Ti plasmid method. Another important requirement for producing useful transformed plants is the availability of an effective plant gene promoter. Many plant gene promoters are known: one very frequently used constitutive promoter is the CaMV (cauliflower mosaic virus) 35-S promoter. However not all such promoters are found to be equally effective in all plants. In some plants, in particular *Compositae*, for example lettuce (*Lactuca sativa*) and sunflower (*Helianthus annuus*), many heterologous constructs are found to have unstable expression levels. Both in primary transformants and in progeny, 'gene silencing' often causes a severe reduction in recombinant gene activity In consequence, the plant reverts to a wild-type phenotype. For practical applications of gene technology this is unacceptable, and presents a real obstacle to the use of recombinant gene technology with plants such as lettuce and sunflower.

According to the present invention, therefore, we provide a method of producing a genetically-modified *Compositae* plant which comprises transforming the plant with a heterologous DNA construct including a DNA sequence adapted to express RNA in the plant under the control of the actin2 (ACT2) gene promoter. We further provide genetically-modified *Compositae* plant cells comprising a heterologous DNA construct including a DNA sequence adapted to express RNA in the plant under the control of the actin2 (ACT2) gene promoter, and *Compositae*, in particular lettuce and sunflower, plants comprising such cells.

The promoter of the actin2 (ACT2) gene derived from *Arabidopsis* has been shown to drive the beta-glucuronidase (GUS) gene in transgenic *Arabidopsis* plants to relatively high levels in vegetative tissues. In the inflorescence a developmental factor seemed to be involved causing a more differentiated expression pattern This is reported by An, Meagher *et al.* (1996), The Plant Journal 10, 107-121: the same reference gives the DNA sequence of the actin-2 promoter (p.109).

The invention will be further described with reference to the drawings, in which:

Figure 1 is a map of a construct pVDH380 comprising the promoter of the actin2 (ACT2) gene derived from *Arabidopsis* arranged to drive the beta-glucuronidase (GUS) gene.

Figure 2 is a map of a construct pVDH641comprising the promoter of the actin2 (ACT2) gene derived from *Arabidopsis* arranged to drive the OXOX gene of wheat;

Figure 3 gives the DNA sequence of the actin2 (ACT2) promoter derived from *Arabidopsis*

Actin is a fundamental cytoskeletal component essential to nearly all eukaryotic cells, in which it forms microfilament structures. There are large families of plant actin genes, with greater diversity than corresponding animal genes. The actin 2 gene promoter is a constitutive promoter to be found in most plants. We particularly prefer to use the actin 2 gene promoter obtained from *Arabidopsis thaliana*: though corresponding actin 2 promoters can readily be isolated from other sources, particularly other plants, and used for the same purpose

The DNA sequence which expresses RNA may be of two main kinds: either a sequence which expresses mRNA which is translated into protein, or a sequence which

produces RNA which is not translated into protein, but which interacts with the biochemistry of the plant cell in another way, for example by inhibiting gene expression.

A preferred use of the present invention is to promote the expression of heterologous genes. However it may also be used to up-regulate or down-regulate the expression of homologous genes. As heterologous genes may be used DNA sequences coding for insecticidal proteins (for example the Bt protein) or fungicidal or antiviral proteins. ACT2 can be used to drive oxox (the oxalate oxidase gene), giving sclerotinia resistance, or other genes like early or late flowering genes (for example ATH1), herbicide resistance genes, insect tolerance genes (aphid resistance in lettuce), virus resistance genes (eg lettuce mosaic virus, LMV), nitrate reductase to lower nitrate content, and genes for increased shelf life Many traits desirable in lettuce and sunflower can be exploited using this promoter.

The use of DNA sequences of homologous genes to inhibit or promote gene expression is quite well understood. A complete gene sequence, under the control of a suitable promoter, that operates effectively in the plant, will generally overexpress the gene product, leading to an amplification of the effect of the protein so produced. Sometimes the gene product is reduced. this phenomenon is termed "co-suppression". Reduction of the gene product is also generally obtained by reversing the orientation of the gene sequence with respect to the promoter so that it produces "antisense" messenger RNA.

A DNA construct for use in the invention may be an "antisense" construct generating "antisense" RNA or a "sense" construct (encoding at least part of the functional protein) generating "sense" RNA. "Antisense RNA" is an RNA sequence which is complementary to a sequence of bases in the corresponding mRNA.

complementary in the sense that each base (or the majority of bases) in the antisense sequence (read in the 3' to 5' sense) is capable of pairing with the corresponding base (G with C, A with U) in the mRNA sequence read in the 5' to 3' sense. Such antisense RNA may be produced in the cell by transformation with an appropriate DNA construct arranged to generate a transcript with at least part of its sequence complementary to at least part of the coding strand of the relevant gene (or of a DNA sequence showing substantial homology therewith). "Sense RNA" is an RNA sequence which is substantially homologous to at least part of the corresponding mRNA sequence. Such sense RNA may be produced in the cell by transformation with an appropriate DNA construct arranged in the normal orientation so as to generate a transcript with a sequence identical to at least part of the coding strand of the relevant gene (or of a DNA sequence showing substantial homology therewith). Suitable sense constructs may be used to inhibit gene expression (as described in International Patent Publication WO91/08299) or a sense construct encoding and expressing a homologous functional protein may be transformed into the plant to over-express the protein.

DNA constructs for use in the invention to inhibit gene expression may comprise a base sequence at least 10 bases (preferably at least 35 bases) in length for transcription into RNA. There is no theoretical upper limit to the base sequence - it may be as long as the relevant mRNA produced by the cell - but for convenience it will generally be found suitable to use sequences between 100 and 1000 bases in length.

As a source of the DNA base sequence for transcription, a suitable cDNA or genomic DNA or synthetic polynucleotide may be used.

The invention will be further described with reference to the following Examples.

EXAMPLES

Lettuce was transformed with various constructs comprising the act2 gene promoter. The act2/GUS construct shown in Figure 1, kindly provided by courtesy of Dr. R. B. Meagher, Dept. of Genetics, University of Georgia, Athens, GA 30602, USA, and by us termed pVDH380 (Figure 1), was used to transform lettuce, in order to evaluate the expression pattern in primary transformants as well as the stability in consecutive generations. The construct pVDH380 was used to make a construct pVDH641 (Figure 2) containing the act2 promoter linked to the oxalate oxidase (OXOX) gene of wheat: this construct was used in transient expression studies in both lettuce and sunflower.

- *Results*

ACT2/GUS in lettuce

The binary vector pVDH380 of Figure 1 contains the NPTII gene as a selectable marker in addition to the ACT2/GUS gene. The act2 promoter sequence includes the first 19 codons of the act2 gene as well as the first exon-intron combination. Vector pVDH380 was used directly to transform lettuce (variety "Evola"), following the Ti plasmid method given in Curtis *et al.* (1994), J. Exp Bot. **45**, 1441-1449.

From this transformation experiment were obtained a total of 38 independent transformants displaying a wide range of GUS-activities (as judged from a histochemical GUS staining using leaf explants of greenhouse grown material). These were compared with control CaMV 35S-GUS transformants, prepared similarly. The act2/GUS transformants of the invention showed higher and more uniform levels of GUS activity than the CaMV controls.

Subsequently, twelve independent act2/GUS transformants were used to carry out further histochemical assays Tissues were taken from leaves, stems, roots, and flowers

(sepals, petals, stamens, carpels) The tissues examined showed consistent GUS activity levels for nine of the events Three of the events however showed a certain degree of variability in expression (e.g enhanced in flowers or vegetative tissues) which is probably due to the site of integration in the genome.

It was concluded that the act2/GUS construct displays a relatively strong, predominantly constitutive expression pattern in transgenic lettuce at the T0 level.

Seeds were harvested and a total number of 15 events were analyzed in the T1 generation using greenhouse grown material. The segregation data in the T1 generation, as well as levels of GUS activity in the T0 generation, are shown in Table 1 below.

TABLE 1

Segregation analysis of transgenic lettuce expressing ACT2/GUS		
Transformant code	*GUS activity in T0*	*Segregation of GUS activity in T1*
Ev-1A-1	+	66+ / 17-
Ev-1A-3	++	20+ / 7-
Ev-2A-2	+	82+ / 24-
Ev-2A-4	-	0+ / 97-
Ev-4A-3	++	75+ / 22-
Ev-5A-2	-	0+ / 102-
Ev-5A-4	-	0+ / 97-
Ev-7A-1	-	0+/ 92-
Ev-8A-1	++	87+ / 22-
Ev-10A-1	+	75+ / 19-
Ev-10A-3	+	58+ / 20-
Ev-12A-1	+	36+ / 13-
Ev-13A-2	++	84+ / 3-
Ev-14A-1	++	78+/ 17-
Ev-14A-2	-	0+ / 98-

"+" = active; "++" = highly active; - = "not detected"

The most important observation is that there is no significant loss of GUS activity when the gene is transferred to the T1. This contrasts sharply with the situation in which

35S-GUS is used which typically results in a total inhibition of gene activity in 90% of the events during transmission from one generation to the next.

EXAMPLE 2

act2/OXOX in lettuce and sunflower

A further experiment was done to illustrate the use of the act2 promoter to drive other genes besides GUS. As the act2/GUS construct pVDH380 (Figure 1) contains 19 codons of the actin2 gene it was decided to modify the promoter by PCR using a primer combination which generates a unique restriction site at the act2 transcription start. This modified promoter was fused to the OXOX gene and inserted into a binary vector, as described below.

Construction of ACT2-OXOX

Starting material for the ACT2-OXOX construct of Figure 2, termed pVDH641 (Figure 2). was the plasmid pVDH380 (Figure 1) which is identical with the plasmid ACT2/GUS described by An et al., cited above.

Figure 1 shows a physical map of the construct pVDH380. In this figure, 'LB' indicates the left border, 'RB' indicates the right border, 'Pnos' indicates the nopaline synthase promoter, 'Tnos' indicates the nopaline synthase terminator, 'NPTII' indicates the neomycin phosphotransferase II gene, 'pACT2' indicates the Actin 2 promoter, 'GUS' indicates the beta-glucuronidase gene and 'KanR' indicates the bacterial kanamycin resistance gene.

The ACT2 promoter was recloned from vector pVDH380 after amplification by PCR.

Figure 3 shows the nucleotide sequence of the Actin 2 promoter region. The sequence corresponding to the forward primer (bold, **Gothic typeface**) as well as to the

complementary sequence (bold underlined) of the backward primer are indicated. The start codon of the Actin 2 gene, ATG is given in bold capitals. In addition, the composition of the forward and backward primers are given. We used a primerset consisting of primer 1 (5'-GC AAGCTT ATT ATG ATC TCA AAT ACA TTG-3') and primer 2 (5'-GC GGATCC TTT ATG AGC TGC AAA CAC AC-3'). Primer 1 contains after the first two nucleotides a HindIII restriction recognition site and subsequently a nucleotide sequence identical to the nucleotide sequence located from position 1358 to position 1379 upstream from the ATG-start codon (see Figure 3). Primer 2 contains after the first two nucleotides a BamHI restriction recognition site and subsequently 20 nucleotides complementary from position 3 to position 22 upstream from the start codon. The DNA fragment which was obtained after amplification was digested with HindIII and BamHI and inserted in the vector pVDH478. pVDH478 is a binary vector containing between the left and right border the NPTII gene, flanked upstream by the nopaline synthase promoter and downstream by the nopaline synthase poly(A)-signal. It also contains the coding region of the oxalate oxidase gene (OxOx) with its own poly(A)-signal which is derived from wheat(for more information on the OxOx gene, including sequence data, see PCT Publication WO92/14824). The resulting vector was called pVDH641. A physical map of pVDH641 is shown in Figure 2. in this Figure, annotations in common with Figure 1 have the same meaning as in that Figure. Additionally, 'TOxOx' indicates the oxalate oxidase terminator, and 'OxOx' indicates the oxalate oxidase gene. The main restriction enzymes are indicated

Sequence analysis confirmed that the ACT2 promoter region had been inserted without any mutation having occurred during the PCR amplification. OxOx activity can be measured in a histochemical assay using oxalate which is converted by the enzyme into a purple dye. The act2/OXOX

fusion showed good levels of OxOx activity in transient assays, using both lettuce and sunflower explants, confirming the functionality of the construct.

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WE CLAIM:

1. A method of producing a genetically-modified *Compositae* plant in which the expression of the modified gene has a reduced tendency to silencing, which comprises transforming the plant with a heterologous DNA construct including a DNA sequence adapted to express RNA in the plant under the control of the actin2 (ACT2) gene promoter.

2. A method as claimed in claim 1 in which the ACT2 gene promoter is derived from *Arabidopsis thaliana*.

3. A method as claimed in claim 2 in which the ACT2 gene promoter has substantially the sequence shown in Figure 3.

4. A method as claimed in any of claims 1-3 in which the RNA to be expressed in the plant codes for the production of a heterologous protein.

5. A method as claimed in any of claims 1-3 in which the RNA to be expressed in the plant codes for the production of a homologous protein.

6. A method as claimed in any of claims 1-3 in which the RNA to be expressed in the plant inhibits the production of a homologous protein.

7. A method as claimed in claim 6 in which the RNA to be expressed in the plant is antisense to DNA coding for a homologous protein.

8. A method as claimed in any of claims 1-7 in which the plant is lettuce or sunflower..

9. Genetically-modified *Compositae* plant cells that may be produced by the process of claim 1 comprising a heterologous DNA construct including a DNA sequence adapted to express RNA in the plant under the control of the actin2 (ACT2) gene promoter.

10. A plant cell as claimed in claim 9 adapted to express RNA that produces recombinant heterologous protein in the cell.

11. A plant cell as claimed in claim 10 in which the heterologous protein is an insecticidal, fungicidal or antiviral protein, or one conferring herbicide resistance.

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16. A vector useful in the process of claim 1 which comprises a DNA construct including a DNA sequence adapted to express RNA in a plant under the control of the actin2 (ACT2) gene promoter, the DNA sequence comprising the gus gene or the oxox gene.

17. *Compositae* plants comprising cells claimed in any of claims 9-15.

18. Plants as claimed in claim 17 which are lettuce.

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GENETIC MODIFICATION OF COMPOSITAE

ABSTRACT

A method is disclosed of producing a genetically-modified *Compositae* plant. The plant is transformed with a heterologous DNA construct including a DNA sequence adapted to express RNA in the plant under the control of the actin2 (ACT2) gene promoter.

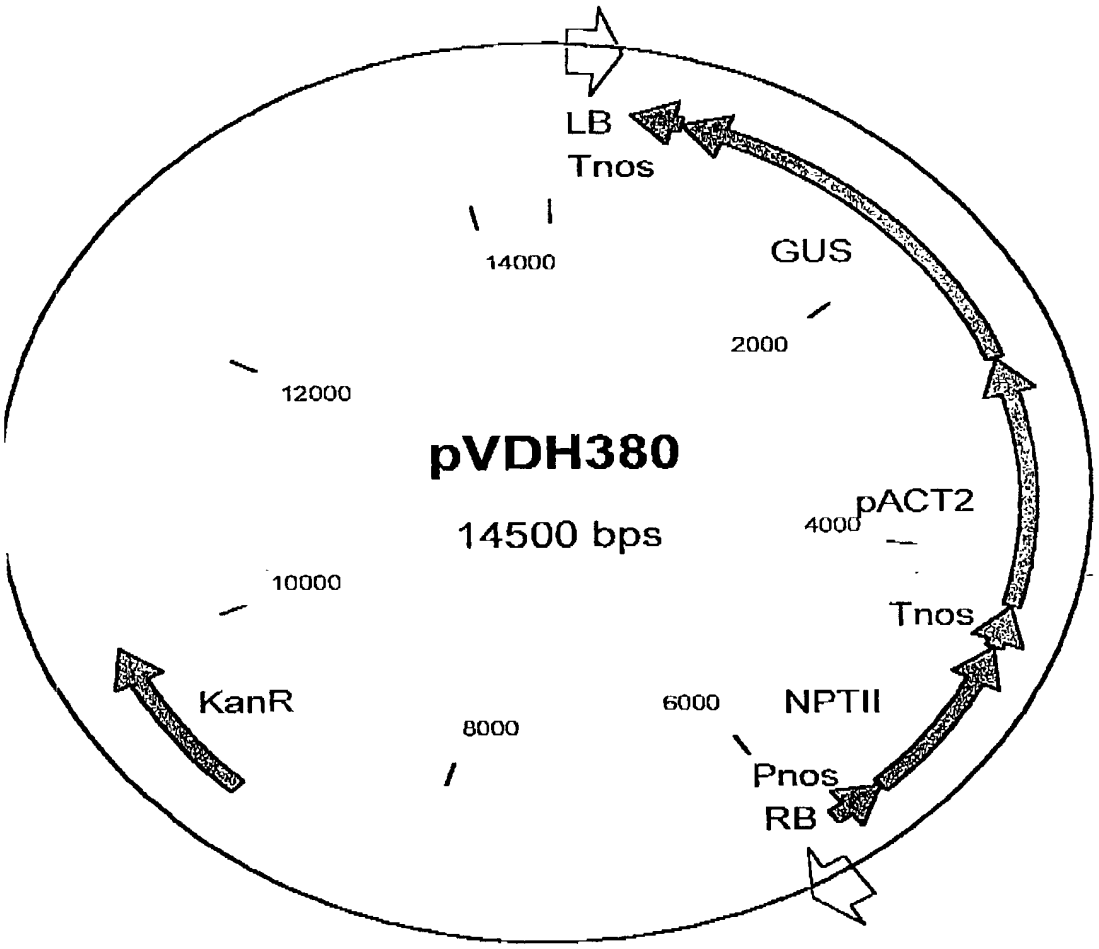
LB
Tnos
GUS
2000
4000
pACT2
Tnos
NPTII
6000
Pnos
RB
8000
KanR
10000
12000
14000
pVDH380
14500 bps

Figure 1

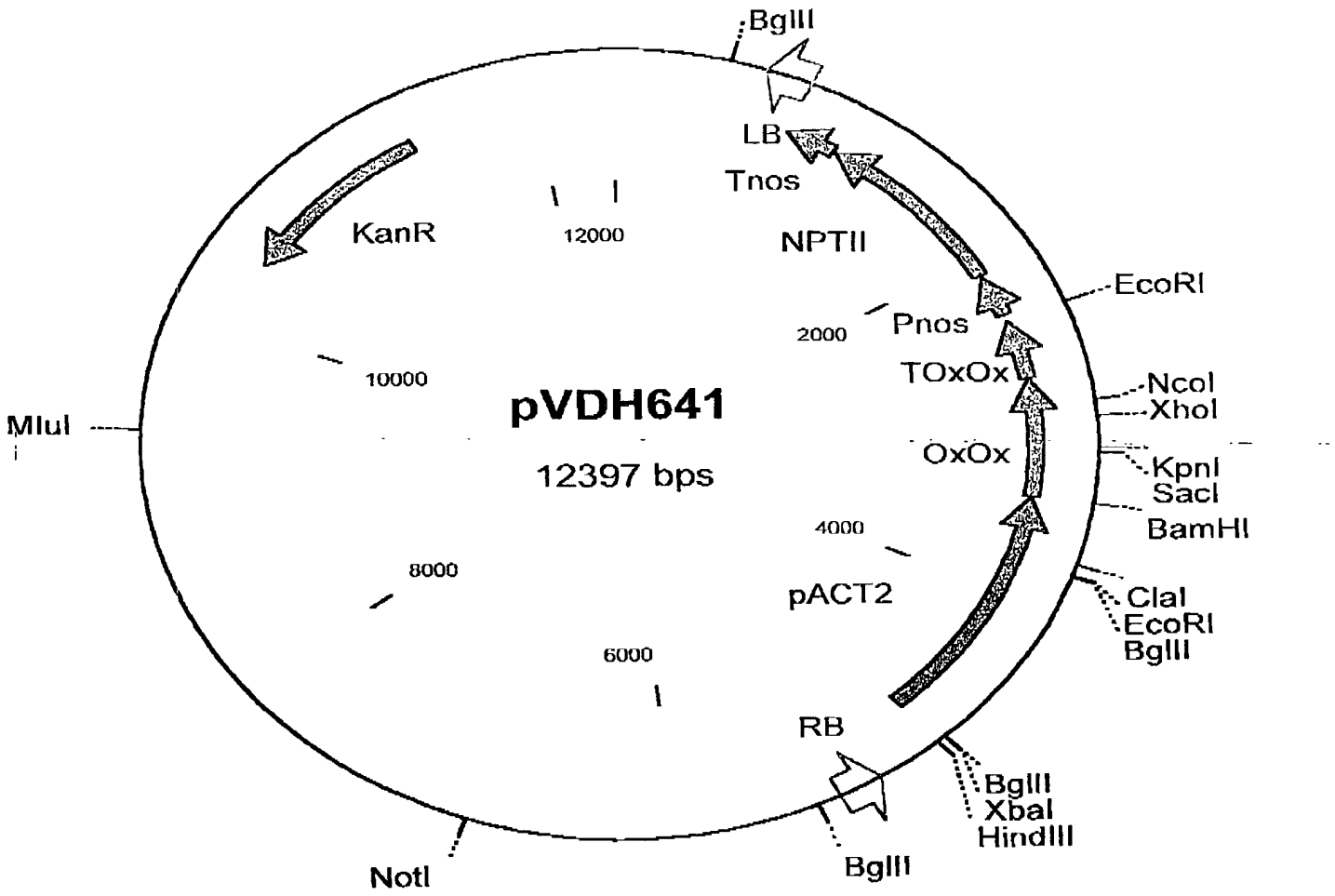
BglII
LB
Tnos
NPTII
KanR
12000
EcoRI
2000
Pnos
TOxOx
10000
NcoI
XhoI
MluI
pVDH641
12397 bps
OxOx
KpnI
SacI
BamHI
4000
8000
pACT2
ClaI
EcoRI
BglII
6000
RB
BglII
XbaI
HindIII
NotI
BglII

Figure 2

```
   1  attatgatct caaatacatt gatacatatc tcatctagat
  41  ctaggttatc attatgtaag aaagttttga cgaatatgnn
  81  acgacaaaat ggctacactc gatgtaattg gtatctcaac
 121  tcaacattat acttatacca aacattagtt agcaaaattt
 161  aaacaactat ttttatgtat gcaagagtca gcatatgtat
 201  aattgattca gaatcgtttt gacgagttcg gatgtagtag
 241  tagccattat ttaatgtaca tactaatcgt gaatagtgat
 281  atgatgaaac attgtatctt attgtataaa tatccataaa
 321  cacatcatga aagacacttt ctttcagggt ctgaattaat
 361  tatgatacaa ttctaataga aaacgaatta aattacgttg
 401  aattgtatga aatctaattg aacaagccaa ccacgacgag
 441  gactaacgtt gcctggattg actcggttta agttaaccac
 481  taaaaaaacg gagctgtcat gtaacacgcg gatcgagcag
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1441  atcc
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HindIII

Actin 2 primer forward. GC AAGCTT **attatgatct caaatacatt g**

BamHI

Actin 2 primer backward GC GGATCC **tttatgagctgcaaacacac**

Figure 3

09868744.010902 #3

PTO/SB/01 (03-01)
Approved for use through 10/31/2002. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☐ Declaration Submitted with Initial Filing OR ☐ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	
First Named Inventor	Schepers
COMPLETE IF KNOWN	
Application Number	09 / 868,744
Filing Date	June 20, 2001
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:

My residence, mailing address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Genetic Modification of Compositae

(Title of the Invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) Dec. 16, 1999 as United States Application Number or PCT International Application Number PCT/GB 99/04317 and was amended on (MM/DD/YYYY) June 20, 2001 (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or (f), or 365(b) of any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent, inventor's or plant breeder's rights certificate(s), or any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	Certified Copy Attached? NO
9828201.5	GB	Dec 21, 1998	☐	☐	☒
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

09868744 .010902

PTO/SB/01 (03-01)
Approved for use through 10/31/2002. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

DECLARATION — Utility or Design Patent Application

Direct all correspondence to: [XX] Customer Number or Bar Code Label 30279 OR [ ] Correspondence address below

Name Garst Seed Company Attn: Dana Rewoldt

Address 2369 330th Street

City	State	ZIP
Slater	IA	50244

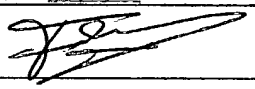
Country	Telephone	Fax
USA	(515) 685-5201	(515) 685-5072

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

NAME OF SOLE OR FIRST INVENTOR : [ ] A petition has been filed for this unsigned inventor

1 - 00

Given Name (first and middle [if any])	Family Name or Surname
Frank	Schepers

Inventor's Signature  Date 8/10/2001

Residence: City	State	Country	Citizenship
Linne NLX		Netherlands	Netherlands

Mailing Address Grotestraat 19

City	State	ZIP	Country
Linne		6067 BP	Netherlands

(3) over

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PTO/SB/02A (11-00)
Approved for use through 10/31/2002. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page ___ of ___

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor		
Given Name (first and middle [if any])		Family Name or Surname	
Cornelis		Van Dun	
Inventor's Signature			**Date** 26 September 2001
Residence: City Roosendaal NLX	**State**	**Country** Netherlands	**Citizenship** Netherlands
Mailing Address Faunaberg 40			
Mailing Address			
City Roosendaal	**State**	**ZIP** 4708 CC	**Country** Netherlands

2-00

over

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PTO/SB/02A (11-00)
Approved for use through 10/31/2002. OMB 0651-0032
U.S. Patent and Trademark Office; U.S DEPARTMENT OF COMMERCE

DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page ___ of ___

Name of Additional Joint Inventor, if any:		☐ A petition has been filed for this unsigned inventor	
Given Name (first and middle [if any])		Family Name or Surname	
Jan		Pertijs	
Inventor's Signature			**Date** 22-09-2001
Residence: City Etten Leur NLX	**State**	**Country** Netherlands	**Citizenship** Netherlands
Mailing Address Achter de Molen			
Mailing Address			
City Etten Leur	**State**	**ZIP** 4873 GX	**Country** Netherlands

3-00

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Pertijs, Jan
Van Dun, Cornelis

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BOX PCT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: F. Scheper et al. Attorney Docket No.: Lettuce

U.S. Application No.: 09/868,744

Int'l. Application No.: PCT/GB99/04317

Int'l. Filing Date: December 16, 1999

Title: GENETIC MODIFICATION OF COMPOSITAE

ASSOCIATE POWER OF ATTORNEY

Seattle, Washington 98101
February 13, 2003

TO THE DIRECTOR – U.S. PATENT AND TRADEMARK OFFICE:

The firm of Christensen O'Connor Johnson Kindness[PLLC] and Lee E. Johnson, Reg. No. 22,946; Gary S. Kindness, Reg. No. 22,178; James W. Anable, Reg. No. 26,827; James R. Uhlir, Reg. No. 25,096; Jerald E. Nagae, Reg. No. 29,418; Dennis K. Shelton, Reg. No. 26,997; Jeffrey M. Sakoi, Reg. No. 32,059; Ward Brown, Reg. No. 28,400; Robert J. Carlson, Reg. No. 35,472; Rodney C. Tullett, Reg. No. 34,034; Daiva K. Tautvydas, Reg. No. 36,077; Maria L. C. Anderson, Reg. No. 40,574; Julie C. VanDerZanden, Reg. No. 38,105; George E. Renzoni, Ph.D., Reg. No. 37,919; Philip P. Mann, Reg. No. 30,960; George S. Farber, Reg. No. 41,497; Kevan L. Morgan, Reg. No. 42,015; John D. Denkenberger, Reg. No. 44,060; and Melanie J. Seelig, Reg. No. 44,328; members of the firm, are hereby granted an associate power of attorney with full power to prosecute the above-identified application and to transact all business in the United States Patent and Trademark Office connected therewith.

Please continue to address all further correspondence relating to this application to the principal attorney:

Dana Rewoldt
Garst Seed Co.
2369 330th Street
Slater, IA 50244

Respectfully submitted,

Dana Rewoldt
Registration No. 33,762
Telephone: 515.685.5201

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS[PLLC]
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

09/868,744

COPY OF PAPERS
ORIGINALLY FILED

DT05 Rec'd PCT/PTO 06 AUG 2002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

RE:	Patent Application for Schepers	:	Date:	July 31, 2002
Serial No.:	09/868,744	:	Group No.:	1661
Filed:	December 16, 1999	:	Examiner:	Shakeel Ahmed
For:	Genetic Modification of Compositae	:		

Assistant Commissioner for Patents
United States Patent and Trademark Office
Washington, D.C. 20231

AMENDMENT

Please amend the specification to include the attached sequence listing information prior to the claims of this application.

Additionally, please amend the specification as follows:

Page 9, line 9, after "combination" insert See Seq. ID No. 2 and Seq. ID No. 3.

A further experiment was done to illustrate the use of the act2 promoter to drive other genes besides GUS. As the act2/GUS construct pVDH380 (Figure 1) contains 19 condons of the actin2 gene it was decided to modify the promoter by PCR using a primer combination, See Seq. ID No. 2 and Seq. ID No. 3, which generates a unique restriction site at the act2 transcription start. This modified promoter was fused to the OXOX gene and inserted into a binary vector, ads described below.

Page 9, line 26, after "promoter region" insert See Seq. ID No. 1.

Page 9, line 27, after "forward primer" insert See Seq. ID No. 2.

Page 10, line 1, after "complementary sequence" insert See Seq. ID No. 3.

Figure 3 shows the nucleotide sequence of the Actin 2 promoter region, See Seq. ID No. 1. The sequence corresponding to the forward primer, See Seq. ID No. 2, (bold, **Gothic typeface**) as well as to the complementary sequence, See Seq. ID No. 3, (bold underlined) of the backward primer are indicated. The start condon of the Actin 2 gene, ATG is given in bold capitals. In addition, the composition of the forward and backward primers are given. We used a primerset consisting of primer 1 (5'-GC AAGCTT ATT ATG ATC TCA AAT ACA TTG-3') and primer 2 (5'-GC GGATCC TTT ATG AGC TGC AAA CAC AC-3'). Primer 1 contains after the first two nucleotides a HindIII restriction recognition site and subsequently a nucleotide sequence identical to the nucleotide sequence located from position 1358 to position 1379 upstream from the ATG-start codon (see Figure 3). Primer 2 contains after the first two nucleotides a BamHI restriction recognition site and subsequently 20 nucleotides complementary from position 3 to position 22 upstream from the start condon. The DNA fragment which was obtained after amplification was digested with HindIII and BamHI and inserted in the vector pVDH478. pVDH478 is a binary vector containing between the left and right border the NPTII gene, flanked upstream by the nopaline synthase promoter and downstream by the nopaline synthase poly(A)-signal. It also contains the coding region of the oxalate oxidase gene (OxOx) with its own poly(A)-signal which is derived from wheat (for more information on the OxOx gene, including sequence data, see PCT Publication WO92/14824). The resulting vector was called pVDH641. A physical map of pVDH641 is shown in Figure 2. in this Figure, annotations in common with Figure 1

have the same meaning as in that Figure. Additionally, 'ToxOx' indicates the oxalate oxidase terminator and 'OxOx' indicates the oxalate oxidase gene. The main restriction enzymes are indicated.

Respectfully submitted,

Dana Rewoldt, Reg. No. 33,762
Advanta USA, Inc.
2369 330th Street, Box 500
Slater, IA 50244
Tel: (515) 685-5201
Fax: (515) 685-5072

CERTIFICATE OF MAILING UNDER 37 C.F.R. 1.8

I hereby certify that the above Amendment and attached postcard are being deposited in with the U.S. Postal Service as first-class mail in an envelope addressed to Assistant Commissioner for Patents, United States Patent and Trademark Office, Washington, D.C. 20231, this 31st day of July, 2002.

Fran T. Tentinger

SEQUENCE LISTING

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PERTIJS, JAN
VAN DUN, CORNELIS

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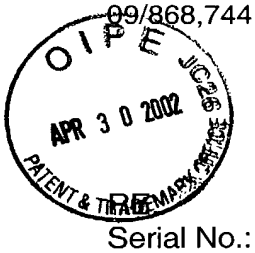

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

RE:	Patent Application for Schepers	:	Date:	April 26, 2002
Serial No.:	09/868,744	:	Group No.:	1661
Filed:	December 16, 1999	:	Examiner:	
For:	Genetic Modification of Compositae	:		

Assistant Commissioner for Patents
Box Sequence
P.O. Box 2327
Arlington, VA 22202

RESPONSE TO NOTIFICATION OF DEFECTIVE RESPONSE

1. This replies to the Office Letter dated April 5, 2002. This Office Letter was received on April 19, 2002 (two weeks after the date of mailing noted in the Office Letter), and the Office Letter did not contain the CRF Diskette Problem Report. Applicant's office called the Examiner on Friday, April 19, 2002, and left a message on the Examiner's voicemail regarding the missing CRF Diskette Problem Report. Applicant's office then received the CRF Diskette Problem Report by telefax on April 23, 2002.

2. Applicant hereby submits with the Notice of Defective Response:

   A. Substitute "Sequence Listing(s)" for the nucleotide and/or amino acid sequence(s) in this application. Each "Sequence Listing" is assigned a separate identifier as required in 37 C.F.R. § 1.82(c) and 37 C.F.R. §§ 1.822 and 1.823.

   B. Substitute copy of each "Sequence Listing" submitted for this application in computer readable form, in accordance with the requirements of 37 C.F.R. §§ 1.821(e) and 1.824.

   C. A statement that the content of each "Sequence Listing" submitted and each computer readable copy are the same, as required in 37 C.F.R. § 1.821(g).

D. Because this submission is made in fulfilling the requirement under 37 C.F.R. § 1.821(g), a statement that the submission includes no new matter.

STATEMENT THAT "SEQUENCE LISTING" AND COMPUTER READABLE COPY ARE THE SAME AND/OR THAT PAPERS SUBMITTED INCLUDES NO NEW MATTER

3. I hereby state that each computer readable form submitted in this application is the same as the "Sequence Listing" to which it is indicated to relate, e.g. the information recorded in the computer readable form is identical to the written sequence listing.

4. All papers accompanying this submission introduce no new matter in the application submitted to the USPTO by the International Bureau.

Please disregard the previously submitted paper copy of the sequence listing as it is written in a previous version of Patentsin.

Respectfully submitted,

Dana Rewoldt, #33,762
Advanta USA, Inc.
2369 330th Street, Box 500
Slater, IA 50244
Tel: (515) 685-5201
Fax: (515) 685-5072

CERTIFICATE OF MAILING UNDER 37 C.F.R. 1.8

I hereby certify that the above Response to Notification of Defective Response, Diskette Containing CRF of Sequence Listing, and attached postcard are being deposited in with the U.S. Postal Service as first-class mail in an envelope addressed to Assistant Commissioner for Patents, Box-Sequence, P.O. Box 2327, Arlington, VA 22202

Mary White

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SCHEPERS, FRANK
PERTIJS, JAN

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SEQUENCE LISTING

<110> VAN DUN, CORNELIS
SCHEPERS, FRANK
PERTIJS, JAN

<120> GENETIC MODIFICATION OF COMPOSITAE

<130> 98102US

<150> PCT/GB99/04317
<151> 1999-12-16

<150> GB 9828201.5
<151> 1998-12-21

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